UNIVERSITY OF PENNSYLVANIA

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STANLEY ELPHINSTONE KERR

A THESIS

IN PHYSIOLOGICAL CHEMISTRY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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STUDIES ON THE INORGANIC COMPOSITION OF BLOOD.

I. THE EFFECT OF HEMORRHAGE ON THE INORGANIC COMPOSITION OF SERUM AND CORPUSCLES.

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INTRODUCTION.

The recent development of methods of analysis for the inorganic constituents of blood, requiring only small quantities of material, has permitted investigation of normal and pathological metabolism of inorganic salts. The fact that blood volume is restored after hemorrhage by the inflow of body fluids, presumably differing in inorganic composition from plasma, suggested that the study of inorganic salt metabolism after hemorrhage should yield information of value. The disturbances in the acid-base relationships after hemorrhage, including changes in the alkaline reserve, also make evident the need for more information concerning the variations in the inorganic ions other than bicarbonate. An investigation was therefore undertaken to determine what changes take place in the inorganic composition of the blood of dogs after single and repeated hemorrhages.

The inorganic composition of plasma differs so widely from that of corpuscles that analyses of whole blood without reference to cell composition and the relative proportion of cells and serum are of little significance. This becomes especially true in a series of analyses of the blood from one animal, because of the decrease in cell volume which results from repeated withdrawals of blood. Although the red blood corpuscles are generally regarded as impermeable to the cations, the evidence is insufficient to warrant the assumption that changes do not occur in the sodium and potassium concentrations in the corpuscles. The necessity of studying

plasma and cells as separate systems has not, however, been recognized in much of the work on the inorganic composition of the blood. In the following experiments on the effect of hemorrhage, serum and corpuscles were separately analyzed.

Methods.

Dogs were used because of the large quantities of blood required for analysis. Blood for analysis or for hemorrhage was always obtained from the heart, without anesthesia, by aspirating the blood through a No. 16 lumbar puncture needle into a 200 cc. cylindrical separatory funnel. The operation of bleeding causes little discomfort to the animal. In the later experiments cocaine (10 mg.) was injected subcutaneously at the point to be punctured, with the result that the animal seldom moved or showed the slightest sign of pain. Even when a large quantity of blood was taken, it was found less disturbing to the dog to take the entire quantity rapidly from the heart rather than more slowly from the jugular vein.

The blood was collected under oil and immediately defibrinated by gentle stirring with a glass rod. Defibrination was preferred to the use of anticoagulants because their effects on shifts of water or ions between corpuscles and plasma are unknown. A little fibrin usually fails to cling to the stirring rod used to defibrinate the blood. In order to remove these loose pieces of fibrin, the blood was next filtered through gauze under oil, and received in a 100 cc. glass cylinder containing a layer of oil. The manipulations were carried on without delay so that no opportunity was given for the corpuscles to sediment appreciably. Before sampling, the blood was stirred by rapidly twisting a long glass rod flattened and bent at the lower end to give the effect of a propeller. This permitted efficient mixing of corpuscles and serum with minimum disturbance of the surface and hence minimum loss of CO_2. The whole blood was then immediately sampled for chloride and water determinations. A rubber stopper carrying a short inlet tube and a long delivery tube was then inserted into the cylinder. By blowing into the short tube, portions of blood were forced out the delivery tube into the specific gravity bottle, the hematocrit tubes and, under oil, into 50 cc. centrifuge tubes.

Separation of Serum.—The oil on the surface of the blood in

the centrifuge tubes was removed as completely as possible and replaced quickly by paraffin just warm enough to be liquid. The tubes were then centrifuged at about 4000 R.P.M. for half an hour. Hematocrit tubes were centrifuged together with the large samples, and the readings found to be constant after half an hour. In the earlier experiments the centrifuging was continued for 1 hour or longer, but this resulted in unnecessary heating of the blood. When removed from the centrifuge the serum was invariably straw-colored and clear, with no trace of hemolysis. The tubes were usually at about body temperature when taken out after half an hour, but hot to the touch when run for an hour. The tubes were cooled in water, the paraffin removed, and the serum aspirated into a small Pyrex Erlenmeyer flask. The final traces of serum were freed from the surface of the corpuscles and the sides of the tube by wiping with strips of filter paper until the corpuscles no longer "wet" the paper. No attempt was made to wash the corpuscles before analysis, for this was found to alter their composition. The corpuscles were analyzed separately for all constituents studied except chlorides which were calculated from the results of analyses on whole blood and serum.

Analytical Methods.

Water was determined in whole blood, serum, and corpuscles by drying 1 cc. portions at 115–120°C. to constant weight for 24 to 48 hours. The samples were measured quickly onto cones of fluted filter paper contained in small weighing bottles which had been dried for at least 24 hours, cooled, and weighed just previous to use.

The *specific gravity* of whole blood and serum was determined at room temperature in a 5 cc. pycnometer.

Weight of Cells: Weight of Blood.—In order to calculate cell chlorides from mM of Cl per 1000 gm. of whole blood and serum, the percentage of cells by weight rather than volume is needed. The percentage by weight may be calculated from the percentage of water in whole blood, serum, and corpuscles as follows:

Let H_2O_{wb}, H_2O_s, H_2O_c represent the percentage by weight of H_2O in whole blood, serum, and cells. Let per $cent_c$ represent the percentage of cells by weight.

Then 100 − per cent$_c$ represents the percentage of serum by weight.

$$100\ H_2O_{wb} = H_2O_s\ (100 - \text{per cent}_c) + H_2O_c \cdot \text{per cent}_c.$$

$$(\text{Per cent}_c) = \frac{100\ (H_2O_s - H_2O_{wb})}{H_2O_s - H_2O_c}.$$

The calculated percentages are recorded in the tables under the heading (weight of cells : weight of blood) 100.

The greatest error of the method rests upon the uncertainty of complete separation of serum from the corpuscles. These weight-hematocrit determinations were checked by cell volume determinations made with the Daland hematocrit, and with specially calibrated long hematocrit tubes which were centrifuged together with the blood samples. The volume hematocrit values were on the average 2.6 per cent higher than the weight hematocrit values, indicating that the packing of the cells is not as great in the large specimens as it is in the narrow tubes.

When blood is defibrinated, more serum than corpuscles is taken out with the fibrin, so that the relative volume occupied by cells in defibrinated blood is greater than that in oxalated blood. When there is an increase of fibrin, as after hemorrhage, this difference becomes greater. The hematocrit readings, therefore, give only approximate ideas of the true cell volume in the circulating blood of the animal, but are accurate for calculating cell composition. Only the weight-hematocrit determinations, calculated as indicated above, are given in the tables.

Chlorides were determined in whole blood and serum by the method of Van Slyke (1923–24). The pipettes used were those with which the samples for water determination were measured, and thus had been calibrated for delivery of both serum and whole blood by weight. The chloride content of the corpuscles was calculated from chlorides in whole blood and serum by the formula developed as follows.

Let Cl_{wb}, Cl_s, Cl_c represent the percentage Cl in whole blood, serum, and corpuscles. Let per cent$_c$ represent the percentage of cells by weight.

Then $100\ Cl_{wb} = Cl_s\ (100 - \text{per cent}_c) + Cl_c \cdot \text{per cent}_c$.

$$Cl_c = Cl_s - \left[\frac{100\ (Cl_s - Cl_{wb})}{\text{per cent}_c}\right].$$

Calcium and magnesium were determined in serum direct, calcium by the method of Clark and Collip (1925). Magnesium

was determined by the colorimetric method of Briggs (1924) in the serum from which calcium had been removed, together with the washings from the calcium precipitate.

Serum proteins were determined by the use of the refractometer, using the methods of calculation of Neuhausen and Rioch (1923) for dog blood.

Sodium, potassium, and inorganic phosphates of both serum and corpuscles were determined in the protein-free filtrates rather than directly in serum or laked corpuscles. Sodium and potassium determinations on serum direct were found unreliable, as will appear in the discussion of these methods. Since it was necessary to remove the proteins from the corpuscles before analysis for sodium and potassium, it was considered best to do the same for serum, handling both alike. The protein-free filtrates were prepared from weighed samples of serum and cells. 20 cc. of serum were weighed in a 100 cc. volumetric flask, diluted with about 50 cc. of water, and the proteins precipitated by the addition of 20 cc. of 20 per cent trichloroacetic acid. After mixing, diluting to the mark, and standing 10 minutes, the contents of the flask were poured into two 50 cc. centrifuge tubes, covered with rubber caps to prevent evaporation, centrifuged for a few minutes, and the almost clear liquid then filtered through ashless filter paper into a Pyrex flask. Due to the much greater proportion of protein in corpuscles than in serum, they were diluted 1:10 instead of 1:5 as for serum. Approximately 15 to 20 gm. of corpuscles were weighed in a 200 cc. volumetric flask, 10 cc. of 0.1 per cent saponin solution and about 100 cc. of water added, the mixture shaken and allowed to stand until hemolysis was complete, as evidenced by the absence of turbidity. One drop of caprylic alcohol was next added to destroy the foam. If this is not done, the protein precipitate occludes many bubbles, and the subsequent dilution to the mark is far from correct. 40 cc. of 20 per cent trichloroacetic acid were next added quickly and the flask rotated and shaken until the precipitation was complete. The precipitate thus formed settled rapidly, and was free from lumps. After all effervescence of gas had ceased, another drop of caprylic alcohol was added and the solution diluted to the mark. After thorough mixing and standing for 10 minutes, the supernatant liquid was filtered through an ashless filter, with precautions to prevent loss

by evaporation, and the filtrate preserved in Pyrex glassware for analysis.

Inorganic phosphates were determined in the serum and cell filtrates by Briggs' modification (1924) of the Bell-Doisy method.

Blood volume was calculated as 8 per cent of the body weight.

Potassium.—The method of Kramer and Tisdall (1921) for the determination of potassium in serum direct was not found to be satisfactory. Determinations on serum direct sometimes agreed with those done on the protein-free filtrate by the method described below, but more often yielded lower results. The sodium cobalti-nitrite reagent precipitates something in serum, probably a protein, which occludes some potassium cobalti-nitrite and prevents its complete oxidation by permanganate. The following procedure for protein-free filtrates, including a modification of the method of washing the precipitate, was found to give accurate results with a solution of known potassium concentration, containing the other inorganic constituents in approximately the concentration found in blood. The method is given in detail, since it differs from that of Kramer and Tisdall in several respects, and because strict adherence to detail is necessary in order to obtain accurate results.

A quantity of protein-free filtrate equivalent to 1 gm. of serum or cells (5 cc. of serum filtrate, or 10 cc. of cell filtrate) is evaporated to complete dryness on the water bath in a platinum dish (or in a small glass beaker). The residue is dissolved in a few drops of water, four or five drops of concentrated HNO_3 are added, and again evaporated to complete dryness. This procedure removes the trichloroacetic acid more effectively than adding a few drops of HNO_3 at the beginning of evaporation. Baking on a hot plate should be avoided as the residue cakes. The dry residue is dissolved in about 0.5 cc. of warm water and the solution transferred to a 15 cc. centrifuge tube, rinsing the dish with several small portions of water, the final volume of solution being 2 cc. When cool, 1 cc. of the sodium cobalti-nitrite reagent as prepared by Kramer and Tisdall (1921) is added *drop by drop* from a burette, mixing thoroughly after each drop, and the tube allowed to stand at least 45 minutes. The solution is diluted to 5 cc. with water and mixed by tapping. A small part of the precipitate at this stage and during washing has a tendency to float on the surface

and no amount of centrifuging will throw it down. The addition of 0.5 cc. of a freshly shaken 1 per cent caprylic alcohol-water mixture before centrifuging will prevent the precipitate from floating. After centrifuging for 10 minutes the supernatant liquid is decanted and the tube placed on a pad of filter paper in the inverted position to drain for a few minutes, taking care not to disturb the precipitate by returning the tube to the upright position before draining. The mouth of the tube is wiped out as completely as possible with the tip of a towel, the sides of the tube washed with 5 cc. of water, and the tip of the tube tapped until the precipitate just begins to mix with the water. Caprylic alcohol suspension is added, and the tube is again centrifuged for 10 minutes. After again decanting and draining for 5 minutes, the washing process is once more repeated (two washings in all), and the titration then made with permanganate as in the regular Kramer-Tisdall method. The decantation and draining of the liquid from the precipitate does not cause loss of the precipitate if the centrifuge tubes are carefully selected. The sides of the narrowing conical portion of the tube must be straight, not curved inward, and the tip should not be broad and shallow.

In connection with the titration itself, the best results are obtained when 2 or 3 cc. of permanganate completely oxidize the precipitate. Whenever there is an abnormally large quantity of potassium present the usual addition of permanganate is found insufficient and one must add sometimes 5 cc. to complete the oxidation. In such cases the results will be found to be too high, and the analyses must be repeated taking an aliquot of such size that 2 or 3 cc. of permanganate will oxidize the precipitate. A curve representing the equivalence of cc. of permanganate to mg. of potassium may be constructed rather than relying on the theoretical calculation.

It is evident that these limitations make the method somewhat unsatisfactory, but strict adherence to the procedure described gave good results. Analysis of a solution of salts in the concentration found in blood gave individual results within 4 per cent of the theoretical, while duplicate determinations nearly always averaged within 2 per cent of the theoretical. It is doubtful whether a macro analysis on 10 cc. of serum by any gravimetric procedure would give any greater accuracy than this micro method

where only 1 cc. is used. Controls were run on a solution of blood salts very frequently, and determinations of potassium were repeated when close checks were not obtained.

Sodium.—Difficulties were encountered in determining sodium in serum by the method of Kramer and Gittleman (1924–25). A detailed study of this method for sodium gave convincing evidence that far more reliable results could be obtained by the use of the protein-free filtrate. Results obtained on serum direct were often 10 per cent lower than those obtained after removal of the proteins. The following method for sodium was found very satisfactory. It is a combination of the methods of Balint (1924) and Kramer and Gittleman (1924–25) with modifications, and is adaptable to serum, whole blood, or cells.

A protein-free filtrate is prepared from serum or cells as already described. An aliquot of the filtrate equivalent to 2 gm. of serum or cells is measured into a platinum dish and evaporated to dryness on the water bath. The residue is moistened with water, five drops of concentrated HNO_3 (iron-free) are added, and again evaporated. (If water is not first added, there will be sufficient effervescence to cause loss of some material.) A few drops of concentrated HNO_3 are now added to the dry residue, and again evaporated. This procedure is repeated once or twice more, and effectively destroys the trichloroacetic acid. The residue is dissolved in about 0.5 cc. of water and transferred to a 50 cc. Pyrex centrifuge tube having a conical bottom, and the dish is rinsed several times until the total volume is 2 cc. A drop of phenolphthalein is added, and the solution made alkaline with a drop or two of 10 per cent KOH (kept in a paraffined bottle). 10 cc. of potassium pyroantimonate, prepared according to the directions of Kramer and Gittleman (1924–25), are next added, and the mixture stirred vigorously with a glass rod, rubbing the sides of the tube until a heavy precipitate forms. If a number of determinations are being done together, the glass rods are numbered to correspond to the tubes. 4 cc. of 80 per cent alcohol are now added from a burette, drop by drop, with constant stirring. The glass rod is rinsed with a small quantity of 30 per cent alcohol contained in a wash bottle. Some of the precipitate remains on the rod. The tube is covered with a rubber cap and allowed to stand for 30 to 45 minutes, not longer. The tube is centrifuged at

moderate speed for 5 minutes, the liquid is decanted and the tube drained in an inverted position for several minutes. After decanting, the tube must be kept in the inverted position, in order to prevent the precipitate from being disturbed. After standing a few minutes, the mouth of the tube is wiped out, and the sides rinsed with 10 cc. of 30 per cent alcohol from a wash bottle. The tube is rotated to wash the precipitate without disturbing it greatly, again centrifuged, the liquid decanted and drained as before. Now each stirring rod is placed in its respective tube, 5 cc. of concentrated HCl (iron-free) are added, dissolving the precipitate adhering to the rod. The packed precipitate is broken up and about 1 cc. of water added. On stirring, the precipitate dissolves instantly, whereas the concentrated HCl will not dissolve it readily. The rod is removed and rinsed with water, and the solution is diluted to 10 cc., mixed, and cooled. About 2 cc. of 2 per cent KI are added, and the liberated iodine is immediately titrated with 0.1 N $Na_2S_2O_3$ till the color is lemon-yellow. About 0.5 cc. of 1 per cent starch solution is added, and the titration continued very slowly to a colorless end-point. The entire process is thus completed in the same centrifuge tube.

Balint (1924) has shown that the precipitate of pyroantimonate occludes some potassium and the results are about 4 per cent higher than theoretical. Hence it is desirable to standardize the whole procedure against a blood salt mixture rather than to standardize the thiosulfate with great accuracy against iodine or biiodate. The thiosulfate is prepared by dissolving the theoretical quantity (24.832 gm.) in water, diluting to a liter. It is protected from the CO_2 of the air, and standardized against pure NaCl, or better against a solution of inorganic salts in the concentration found in blood. A series of determinations of sodium is made on 2 cc. portions of such a solution in the manner described. The sodium equivalent of each cc. of thiosulfate is then calculated as follows:

Mg. Na in sample taken ÷ cc. $Na_2S_2O_3$ used = mg. Na per cc. $Na_2S_2O_3$.

The factor thus obtained should not differ by more than 4 per cent from the theoretical factor 1.15.

Attention should be called to several minor points. (*a*) The centrifuge tubes and glass rods must be seasoned as described by

Balint by using them in several precipitations of sodium before attempting accurate work. They must be cleaned with water only, never with soap or cleaning fluid. (*b*) When the reagent fails to precipitate the sodium the fault may be in not having destroyed the trichloroacetic acid properly. If properly conducted, one drop of potassium hydroxide will render the solution alkaline. (*c*) Traces of dissolved glass will interfere with the precipitation of sodium pyroantimonate. Hence in preparing the pyroantimonate reagent, paraffined funnels and flasks should be used from the time the KOH has been added until the reagent is finally stored in a paraffined bottle. (*d*) When the end-point of the titration is reached, if the blue color immediately reappears, and continues to do so as more thiosulfate is added, indicating a slow liberation of iodine, the reagent is unsatisfactory and must be discarded.

Kramer and Gittleman (1924–25) recommend adding 3 cc. of 95 per cent alcohol, distilled over KOH. If not distilled, the alcohol forms a precipitate on adding it to the reagent. This also occurs if the alcohol is added too rapidly. 80 per cent alcohol was found satisfactory without redistillation and does not form a precipitate in the reagent as readily as 95 per cent alcohol, by causing too great a concentration of alcohol at the point of addition. A test should, of course, be made to see that no precipitate is formed when 4 cc. of the 80 per cent alcohol are added drop by drop to 10 cc. of the reagent.

This method has given excellent results in the hands of several workers in this laboratory. It is to a certain extent empirical, and details must be strictly adhered to. It has the advantage of eliminating much of the personal equation, since each worker standardizes not only the solutions but the entire procedure on a solution of known sodium content. In fact one cannot rely on sodium determinations unless such controls on a known sodium solution are frequently made.

Results.

The experimental results may be grouped under three heads. (1) The changes in blood 24 hours after a large hemorrhage (Experiments 1-H-A, 1-H-B, and 2-H, Tables I and II). (2) The changes in blood after several days of repeated hemorrhage (Ex-

periments 4-H and 5-H, Tables III and IV). (3) Control experiments—the effect of fasting (Experiments 1-F and 2-F, Tables V and VI).

Experiments 1-H-A and B were conducted on the same dog, 8 days apart. In both cases the dog was offered food, but would not eat for 24 hours after a hemorrhage. In Experiment 2-H the dog fasted before and during the experiment. In these three experiments the hemorrhage was a severe one. The first portion of blood drawn was used for the initial analysis; samples were taken from the animal 4 to 6 hours later, and again 24 hours after the hemorrhage. In Experiments 1-H-A and B the intermediate samples were discarded.

Tables I and II show the absence of any great changes in the inorganic composition of either serum or cells. There is a slight fall in serum sodium in Experiments 1-H-A and B and in 2-H. There is a fall in chlorides in Experiments 1-H-B and 2-H. In the red corpuscles in Experiment 1-H-B there is a decrease in sodium and a rise in potassium following the hemorrhage. These changes are beyond the limits of error of the determinations, and cannot be accounted for by shifts of water. Similar variations, though less pronounced, occurred in Experiment 2-H. It is thus seen that a single hemorrhage produces only a slight fall in sodium and in chlorides in serum, and perhaps a slight fall of sodium and rise of potassium in the corpuscles. In each of these three experiments the fall in hematocrit values indicates that tissue fluids had entered the blood causing a considerable dilution by the end of 24 hours.

Having failed to find any great changes after a single hemorrhage, it was decided to investigate the effects of repeated hemorrhage. Two experiments were conducted in which fasting dogs were bled daily, each hemorrhage being a severe one. In Experiment 4-H on the 4th day of severe hemorrhage, when the bleedings had removed a quantity of blood nearly equal to the original blood volume of the animal, it was found that the sodium and chloride concentrations of the serum had diminished, decreasing in equivalent quantities. A normal value was obtained for serum potassium after repeated hemorrhage. The original potassium level was abnormally low in both serum and cells, but the analyses were repeated, leaving no doubt as to their accuracy. In the cells, sodium was unchanged, but potassium increased greatly in concentration.

TABLE I.

Experiment 1-H. Effect of Hemorrhage.

Dog 1, male; weight 21.5 kilos. Calculated blood volume 1720 cc. Received water daily.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Weight of cells / Weight of blood × 100.	Remarks.	Analyses.		Sample I-A.	Sample II-A.	Sample I-B.	Sample II-B.
			cc.			Fasted Jan. 18.						
I-A	Jan. 19	10 a.m.	320	19	47	Refuses food.	Sp. gr.	Whole blood.	1.061	1.051	1.049	1.045
	" 19	3 p.m.	180	10				Serum.	1.025	1.022	1.025	1.022
							H_2O, per cent.	Whole blood.	79.3	82.2	82.6	85.4
								Serum.	92.2	93.1	92.0	93.3
								Cells.	66.1	66.1	67.4	66.2
II-A	" 20	11 a.m.	120	7	40	Dog is fed during week following Experiment II-A.	Na mM per 1000 gm.	Serum.	153	148	146	135
								Cells.	117	118	114	108
							K " " 1000 "	Serum.	5.1	5.2	5.6	5.4
								Cells.	8.2	8.7	8.3	10.0

I-B	Jan. 28	11 a.m.	635	37	38	Refuses food.	Ca mM per 1000 gm.	Serum.	2.9	2.1	3.7	2.9
	" 28	4.15 p.m.	120	7			Mg " " 1000 "	"	1.0	0.9	0.9	0.7
II-B	" 29	11 a.m.	120	7	29		Cl " " 1000 "	Whole blood	Lost.	88	88	89
								Serum.	102	110	109	104
								Cells.		56	55	54
							P (inorganic) mM per 1000 gm.	Serum.	1.1	1.2	1.3	1.1

TABLE II.

Experiment 2-H. Effect of Hemorrhage.

Dog 2, female; weight 13.9 kilos. Calculated blood volume 1112 cc. Last fed Feb. 6; fasted throughout experiment. Given water daily.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Weight of cells / Weight of blood × 100.	Analyses.		Sample I.	Sample II.	Sample III.
			cc.			Sp. gr.	Whole blood.	1.057	1.049	1.040
							Serum.	1.024	1.021	1.021
I	Feb. 11	10 a.m.	425	38	47	H_2O, per cent.	Whole blood.	80.2	82.2	86.2
							Serum.	92.5	93.9	93.7
							Cells.	66.3	67.6	66.8
						CO_2 mM per 1000 cc.	Serum.	18.9	21.0	17.7
						pH (electrometric).	"	7.34	7.32	7.35
						Protein (Kjeldahl), per cent.	"	6.22	5.13	5.26
							Cells.	33.97	32.67	32.41
II	" 11	2.30 p.m.	175	16	44	Na mM per 1000 gm.	Serum.	136	134	135
							Cells.	107	106	102

III	Feb. 12	10 a.m.	175	16	28	K mM per 1000 gm.	Serum.	4.7	4.8	5.2
							Cells.	6.8	8.3	7.2
						Ca " " 1000 "	Serum.	2.7	2.7	2.3
						Cl " " 1000 "	Whole blood.	84	85	89
							Serum.	109	107	100
							Cells.	56	57	61
						P(inorganic) mM per 1000 gm.	Serum.	1.0	1.5	Lost.

TABLE III.

Experiment 4-H. Effect of Repeated Hemorrhage.

Dog 1, male; weight 21.4 kilos. Calculated blood volume, 1712 cc. Last fed June 7; fasted throughout experiment. Received water daily.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	$\frac{\text{Weight of cells}}{\text{Weight of blood}} \times 100$.	Analyses.		Sample I.	Sample II.
			cc.						
I	June 9	10 a.m.	550	32.4	52	Sp. gr.	Whole blood.	1.059	1.042
							Serum.	1.025	1.020
	" 10	10 "	335	20		H_2O, per cent.	Whole blood.	79.1	85.0
							Serum.	93.0	93.3
	" 11	12 m.	425	25			Cells.	66.5	65.8
II	" 12	10 a.m.	350	21	30	Na mM per 1000 gm.	Serum.	145	136
							Cells.	102	102
	" 13	11 "	450	26.5		K " " 1000 "	Serum.	3.8	5.4
							Cells.	4.9	8.4
						Ca " " 1000 "	Serum.	2.8	2.4
						Mg " " 1000 "	"	0.4	0.6

TABLE IV.

Experiment 5-H. Effect of Repeated Hemorrhage.

Dog 6, male; weight 22.0 kilos. Calculated blood volume 1760 cc. Received water daily.

Sample No	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Weight of cells / Weight of blood × 100.	Remarks.	Analyses		Sample I.	Sample II.	Sample III.	Sample IV.	Sample V.
			cc.										
I	June 17	10–11 a.m.	750	43	56	Fasted 3 days before, and during experiment.	Sp. gr.	Whole blood.	1.063	1.043	1.044	1.044	1.035
								Serum.	1.027	1.021	1.024	1.022	1.020
	" 18	10 a.m.	400	23			H_2O, per cent.	Whole blood.	76.6	85.4	84.2	84.3	87.8
								Serum.	91.5	92.8	92.3	92.3	93.7
								Cells.	64.8	65.6	65.4	66.9	68.4
	" 19	10.30 a.m.	200	12	37	Gave 300 cc. water by stomach tube.	Na mM per 1000 gm.	Serum.	141	118	131	126	138
								Cells.	104	102	93	96	105
	" 19	4 p.m.	160	9		Gave 300 cc. H_2O.							
	" 20	10.30 a.m.	400	23	23								

II	June 22	10.30 a.m.	375	21	27		K mM per 1000 gm.	Serum.	5.8	4.8	6.3	4.9	4.8
								Cells.	6.5	9.8	15.2	11.9	10.1
III	" 24	11 "	150	9	30	Gave 300 cc. water by stomach tube. Weak.	Ca " " 1000 "	Serum.	3.1	2.6	2.8	2.7	2.9
							Mg " " 1000 "	"	0.5	0.4	0.7	Lost.	0.4
IV	" 27	9 "	150	9	31	Weight 18.6 kilos. Began to feed at noon, and fed daily hereafter.	Cl " " 1000 "	Whole blood.	99	82	80	80	96
								Serum.	106	89	92	92	106
								Cells.	93	65	42	53	62
V	" 30	9.30 "	150	9	24	Condition good.	P (inorganic) mM per 1000 gm.	Serum.	1.0	1.2	1.7	1.7	1.0
								Cells.	0.7	1.5	2.3	2.2	2.3
							Protein, per cent.	Serum.	7.1	5.0	6.3	6.1	5.0

TABLE V.

Experiment 1-F. Effect of Fasting.

Dog 7, weight 12.0 kilos. Calculated blood volume 960 cc. Fed once a day for a week previous to experiment; began fasting July 20. Received water daily.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Weight of cells / Weight of blood × 100.	Analyses.		Sample I.	Sample II
			cc.						
I	July 20	10 a.m.	150	16	41	Sp. gr.	Whole blood.	1.056	1.049
							Serum.	1.024	1.023
						H_2O, per cent.	Whole blood.	81.0	82.8
							Serum.	91.4	93.1
							Cells.	66.2	67.0
II	" 27	10 "	200	21	39	Na mM per 1000 gm.	Serum.	139	136
							Cells.	108	109
						K " " 1000 "	Serum.	4.6	5.0
							Cells.	8.7	10.0
						Ca " " 1000 "	Serum.	3.0	2.7
						Mg " " 1000 "	"	0.9	0.9

Cl mM per 1000 gm.	Whole blood.	84	80
	Serum.	103	103
	Cells.	58	45
P (inorganic) mM per 1000 gm.	Serum.	1.3	1.3
	Cells.	0.9	1.1
Protein, per cent.	Serum.	6.4	5.9

TABLE VI.

Experiment 2-F. Effect of Fasting.

Dog 8, female; weight 16.6 kilos. Calculated blood volume 1328 cc. Fed once a day for a week previous to experiment; began fasting July 22. Received water daily.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	$\frac{\text{Weight of cells}}{\text{Weight of blood}} \times 100$.	Analyses.		Sample I.	Sample II.
			cc.			Sp. gr.	Whole blood.	1.058	1.061
							Serum.	1.025	1.025
I	July 22	10 a.m.	150	11	49	H_2O, per cent.	Whole blood.	79.3	78.8
							Serum.	91.8	92.2
							Cells.	66.2	66.3
II	" 29	10 "	100	7	51	Na mM per 1000 gm.	Serum.	135	134
							Cells.	113	114
						K " " 1000 "	Serum.	5.1	5.0
							Cells.	9.0	8.9
						Ca " " 1000 "	Serum.	3.0	3.1
						Mg " " 1000 "	"	0.9	1.2

Cl mM per 1000 gm.	Whole blood.	78	77
	Serum.	103	106
	Cells.	53	50
P (inorganic) mM per 1000 gm.	Serum.	0.9	1.1
	Cells.	1.9	1.9
Protein, per cent.	Serum.	6.5	6.7

In Experiment 5-H, on the 6th day, after 4 days of heavy blood losses and 1 day of recuperation, the changes observed were very similar to those of Experiment 4-H; *i.e.*, a fall of sodium and chloride in serum, a rise of potassium in cells. On the 6th day the dog was again bled heavily, and the next analysis made 2 days later. Although fasting, sodium and chloride increased sharply in the serum, but decreased in the corpuscles. Both potassium and inorganic phosphate increased remarkably in the cells, and inorganic phosphate increased in serum. The period of recovery was next studied in this dog, taking minimum blood samples (Nos. IV and V, Table IV) 3 days apart. In the 3 day recovery period without food, there were no changes of significance. The dog at this time was in good condition. After 3 more days with food, the serum had returned to its original normal composition, except for proteins. In the cells, potassium and inorganic phosphate remained at a high level, while sodium became normal.

The determinations of inorganic phosphates in the cells of Experiments 1-H-A and B and 2-H were discarded because they were high and variable, presumably due to overheating while centrifuging. Buell (1923) found no inorganic phosphate in corpuscles, and believes the commonly found inorganic phosphate in corpuscles is due to hydrolysis of organic phosphorus compounds. In experiments 4-H and 5-H, however, the time of centrifuging was exactly ½ hour, so that the increase in inorganic phosphate in Experiment 5-H is thought to be of significance.

The control Experiments 1-F and 2-F were undertaken to determine whether or not 1 week's fasting alone might cause changes in the inorganic composition of blood. Tables V and VI show practically no change in any of the ions in either serum or corpuscles.

In spite of all the care taken in the determination of chlorides in whole blood and serum, the calibration of the pipettes against each serum and blood, and the careful measurement of weight hematocrit, we are not satisfied with the calculated concentration of chlorides in cells. The variations in the chloride ratio between serum and cells are not those expected from the changes in pH known to occur after hemorrhage. In two cases the ratios are greater than unity, which is obviously incorrect, according to our knowledge of the distribution of chlorides governed by the Donnan equilibrium.

For the purpose of studying the osmotic relationships, all the data of the accompanying tables were recalculated as millimols per kilo of water, but as no new information was gained, the tables were omitted.

DISCUSSION.

After severe hemorrhage the organism employs every possible means to secure normal nutrition of the tissues by restoring blood volume. The dependence of a normal blood flow upon the restoration of blood volume has been shown by Gesell, Capp, and Foote (1922–23). That the inflow of tissue fluids is an important factor in restoring blood volume after hemorrhage has long been recognized. In an investigation of the changes which take place in the chemical composition of the blood after hemorrhage, it becomes necessary therefore to learn something of the nature of the tissue fluids. Furthermore such an investigation naturally involves the factors which control the normal composition and volume of plasma. Among these may be mentioned the activity of the kidney in governing to a great extent plasma volume as well as its content of salts. Other factors of great importance are the permeability of the capillaries, and the passage of fluids and ions through their walls. Changes in blood pressure, osmotic pressure, and in the protein concentration of the plasma and lymph may influence these factors in various ways.

It is important to know the effects of hemorrhage on these various mechanisms. It has been observed by various investigators that hemorrhage may reduce blood pressure sufficiently to stop or retard the excretion of urine. When the blood pressure is raised by inflow of fluids from the tissues or gastrointestinal tract, urine excretion again begins. The cessation of urine flow after severe hemorrhage therefore aids the organism in restoring blood volume by conserving both water and salts, but limits its ability to regulate the concentration of the individual ions.

The passage of fluids and ions through capillary walls is an important mechanism for regulating plasma volume and composition, as already stated. That tissue fluids actually pass into the blood stream after severe hemorrhage and consequent lowering of blood pressure is unquestioned, although the mechanism by which it occurs is not well understood (Krogh, 1922). Al-

though there may be delay in the dilution of the blood, if the animal lives blood volume eventually rises due to inflow of these fluids. Carrier, Lee, and Whipple (1922) found that after hemorrhage the dilution of the blood is complete in 18 to 24 hours. Robertson and Bock (1919) on the other hand believe that without forced ingestion of water, blood volume may remain abnormally low for days. They observed that the organism retains great quantities of ingested water after hemorrhage until blood volume is fully restored. Lee, Carrier, and Whipple (1922) state that copious water ingestion may even raise the plasma level of a normal animal 10 to 40 per cent above normal. The fact that water given by mouth or rectum may aid in the restoration of blood volume indicates the necessity of considering the absorption of fluids from the gastrointestinal tract as well as the inflow of tissue fluids in the dilution of the blood after hemorrhage. It seems likely that the response of the body to hemorrhage in restoring blood volume may differ widely according to the availability of fluids from the various sources. In the absence of excess water in the gastrointestinal tract it is evident that the initial dilution of the blood must be caused by inflow of the extracellular fluid or lymph, which is in a state of constantly changing equilibrium with plasma on the one hand and with the intracellular fluids on the other. As blood volume is restored, the extent to which intracellular fluids are drawn into the blood depends upon the quantity of lymph which can be drained from the tissue spaces, and upon the extent to which the intracellular fluids compensate for the loss of lymph. An abundant water supply in the gastrointestinal tract should certainly have a sparing action on the use of the intracellular fluids.

That intracellular fluids are actually drawn upon is shown by Gamble, Ross, and Tisdall (1923) in their study of the metabolism of fixed base during fasting. They found that in two cases of fasting children, five to six times as much intracellular as extracellular body water was lost. They furnished evidence that the destruction of cellular protoplasm resulted in the passage of a proportionate amount of the cellular water and salts into the blood, and thence into the urine. These facts lead us to expect that a considerable proportion of the tissue fluids entering the blood after hemorrhage should be intracellular fluids with their salts. That

cellular protoplasm is actually destroyed is indicated by the increased nitrogen metabolism occurring after hemorrhage. Hawk and Gies (1904) found an increased excretion of nitrogen in the urine, Taylor and Lewis (1915) found an increase in the urea and non-protein nitrogen of the blood, and György and Zunz (1915) noted an increase in the amino acids of the blood. That there is great destruction of tissue after hemorrhage is also evident from the loss of weight.

If lymph, intracellular fluids, and fluid absorbed from the gastrointestinal tract differ appreciably from each other and from plasma in composition, it should be possible by investigating what changes occur in the blood after hemorrhage to determine the nature of the diluting fluids and thus their origin. Such an investigation becomes of interest in view of the hypothesis of Milroy (1917) that the fluids entering the blood after hemorrhage are hypotonic. It was hoped that this research would throw light on this point, and would aid in determining whether or not the intracellular fluids with their peculiar inorganic composition enter the blood after hemorrhage, and change its composition.

It is evident, therefore, that a knowledge of the composition of both extracellular and intracellular fluids is necessary. The literature on this subject is very meager. The only analyses of dog lymph found were those of Petersen and Hughes (1925), but their figures are so variable that the analyses of human lymph by Munk and Rosenstein (1891) are given. The inorganic composition of human, horse, and dog lymph appears to be very similar, judging from the few analyses available. The analyses of dog muscle are those of Katz (1896). The average of the analyses of the normal serum and corpuscles of the dogs studied in this and the following paper are given (see Table VII).

The data of this table give us a basis for discussing the changes found in the blood after hemorrhage. It is evident that if the dilution of the blood is accomplished only by absorption of water from the gastrointestinal tract, there should result a decrease in all the inorganic constituents and protein of the serum, assuming that this liquid would have a low salt content. Dilution by lymph only should result in a decrease in serum potassium and possibly protein. Intracellular fluid only should produce a decrease in sodium and chloride, and an increased concentration of potas-

sium and magnesium. It is obvious that both lymph and fluid from the gastrointestinal tract aid in restoring blood volume, and the question is merely whether intracellular fluids are actually drawn upon. The results of dilution of the blood by intracellular fluids combined with either one of the other two fluid sources are self-evident. We actually find on analysis of serum a slight fall of sodium after a single hemorrhage, and a greater decrease after repeated hemorrhage. Potassium, magnesium, and calcium however remain unchanged. A normal potassium level after hemorrhage can only be accounted for by a partial dilution with intracellular fluids. That sodium and chlorides decrease is confirmatory evidence. The inflow of intracellular fluids in the proportion that may be assumed from the work of Gamble, Ross, and Tisdall (1923) should however cause an increased concentration

TABLE VII.

Comparison of the Inorganic Composition of Muscle, Lymph, Serum, and Corpuscles.

Substance.	H_2O	Na	K	Ca	Mg	Cl
	per cent	*mM per 1000 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*
Dog muscle.....	76.4	41	83.4	1.75	9.87	22.8
Human lymph.....		140	3.17	2.72	1.03	101
Dog serum.....	92.5	141	5.19	2.90	0.82	106
" corpuscles.....	66.1	109	7.77			55.8

both of potassium and of magnesium that could not escape detection, were it not for the fact that the excess of these cations may be excreted by the kidney or stored by some other tissue. For this reason it is significant that the potassium concentration in the corpuscles increases remarkably after repeated hemorrhage. The total evidence indicates that a considerable part of the dilution of the blood is due to intracellular fluids.

The most surprising change in blood composition after hemorrhage is however not in serum, but in the corpuscles. In each of the experiments recorded in the accompanying tables there were observed changes in the potassium content of the corpuscles. The changes were small after a single hemorrhage, and large after repeated hemorrhage. In Experiments 1-H-B, 4-H, and 5-H the

changes were far beyond the limits of experimental error of the determinations, and were too great to be accounted for by changes in the water content of the corpuscles. In Experiment 5-II, for example, the potassium concentration rose from 6.5 mM to 15.2 mM per 1000 gm. of cells, while the water content actually increased from 64.8 to 65.4 per cent.

These changes point to a possible permeation of the corpuscle wall by potassium ions. Frequent references are found in the literature to the impermeability of the red blood corpuscle to cations. It has been shown by Doisy and Eaton (1921) that variations in CO_2 do not cause a shift of sodium and potassium between plasma and corpuscles, which earlier workers believed took place. Ashby (1924) found red blood corpuscles permeable to potassium in aqueous salt solutions. Their permeability to sodium was much less than to potassium.

The variations of sodium in the corpuscles are also interesting. In Experiments 1-H-B and 2-H the fall of sodium is only a little beyond the experimental error. In Experiment 4-H there is no change, but in Experiment 5-H sodium decreased in the corpuscles far beyond the limits of error. In this experiment the low sodium in the corpuscles parallels the lowered concentration of sodium in the serum, and suggests a loss of sodium from the corpuscles.

In order to determine whether ions pass in and out of corpuscles it is necessary to recalculate all of the results, correcting for water shifts. These calculations were made, and as the observed water shifts were small, the relationships were not changed. Therefore the data are not presented although they may be calculated from the tables.

It has already been pointed out that repeated hemorrhage causes a passage of intracellular tissue fluids, potassium-rich and sodium-poor, into the blood stream. Although the lowered level of sodium is shared by both corpuscles and serum, at least in Experiment 5-H, increases in cell potassium are not paralleled by any changes of potassium in the serum. The corpuscles seem to be exhibiting a selective action for potassium although there is no information available to suggest why there should be increased selective action after hemorrhage. The selective action of tissues in general for potassium is seen in the high potassium content of muscle, and in the fact that corpuscles contain more potassium than the serum in which they are suspended.

In searching for an explanation of the high cell potassium, the possibility of the formation of new corpuscles rich in potassium must be considered. A single hemorrhage will cause a sudden washing into the blood stream of the reserves of red cells in the bone marrow. This does not influence the average corpuscle composition as observed 24 hours after hemorrhage. The reserves therefore appear to be no higher in potassium concentration than the cells already in the blood stream. Repeated hemorrhage should produce the maximum stimulus to rapid formation of new red cells, and it is after repeated hemorrhage that the rise in cell potassium is observed. At this time the body has been depleted of its sodium, while excessive quantities of potassium are being set free by destruction of cellular protoplasm. It is thus possible that the organism makes use of the more available potassium in forming new corpuscles. The high potassium values in corpuscles after repeated hemorrhage may possibly be associated with the disturbances which occur in serum and cell proteins. Foster and Whipple (1921–22) have shown that the concentration of fibrinogen in plasma may fluctuate widely after hemorrhage, often greatly exceeding its normal concentration. Inagaki (1907) noted marked changes in the albumin-globulin ratio in the serum of rabbits on the days following severe hemorrhage. The proportion of globulin to albumin was greatly increased, although the total serum proteins were diminished. Inagaki also observed that after hemorrhage the red corpuscles became smaller, and lost a protein which he believed was globin. Scott (1915–16) confirmed this observation by finding that a protein passed from corpuscles to serum when blood was diluted with Ringer's solution. Serum proteins were determined in Experiments 2-H, 4-H, and 5-H, and were found to decrease after hemorrhage in each experiment. In Experiment 2-H the total nitrogen of corpuscles was determined by the Kjeldahl method, and a definite decrease was found, confirming the observations of Inagaki and Scott. It is difficult to associate a loss of protein from the cells with a gain in potassium.

In a discussion of variations of the inorganic constituents of blood it would seem that the theories of Milroy (1917) and of Henderson and Haggard (1922) should be mentioned. Milroy observed after hemorrhage a diminished specific gravity of whole blood, and a diminished alkaline reserve of plasma, which was

slightly hemolyzed. He concluded that hypotonic tissue fluids low in bicarbonate entered the blood after hemorrhage. The lowered specific gravity of whole blood was undoubtedly mainly due to diminished concentration of cells. The diminished cation and protein concentrations found in the present work indicate that the osmotic pressure of the blood is probably lowered after hemorrhage, though no hemolysis was ever observed. Henderson and Haggard (1922) propose the hypothesis that the low alkaline reserve after hemorrhage is due to diffusion of base from the blood into the tissues. The changes in base necessary to account for maximum changes in alkaline reserve are so small compared with the total base of blood that the variations might be within the errors of our experiments. But considering the low alkaline reserve immediately after hemorrhage and the high alkaline reserve shown to occur later by Evans (1921) and Bennett (unpublished), the fact that sodium and chloride rise and fall in equivalent quantities suggests that the diffusion of base as a bicarbonate salt in and out of the blood does not account for the variations.

SUMMARY.

1. The effects of hemorrhage on the inorganic composition of serum and corpuscles have been studied.

2. Some modifications of existing methods of analysis for the inorganic constituents of serum and corpuscles are described.

3. A single hemorrhage, amounting to from one-third to one-half of the total blood volume results in a slight decrease in both sodium and chloride of serum, and no significant change in corpuscles.

4. Repeated severe hemorrhage produces a greater fall of sodium and chloride of serum, and an increase in the inorganic phosphate. No changes in the potassium, calcium, or magnesium of serum were observed. A large increase in the potassium concentration of the corpuscles occurred.

5. Possible causes for the high cell potassium are discussed, but the experimental data are insufficient to warrant any conclusions.

The writer desires to express to Dr. D. Wright Wilson, who suggested this study, his sincere thanks for many valuable suggestions, and much helpful criticism in both this and the following paper.

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STUDIES ON THE INORGANIC COMPOSITION OF BLOOD.

II. CHANGES IN THE POTASSIUM CONTENT OF ERYTHROCYTES UNDER CERTAIN EXPERIMENTAL CONDITIONS.

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INTRODUCTION.

The red corpuscle has been generally regarded as impermeable to the cations sodium, potassium, calcium, and magnesium. In the preceding paper (Kerr, 1926), however, it has been shown that the concentration of potassium in the red cells of the dog increases after repeated hemorrhage. The fact that no satisfactory explanation for this change could be found suggested a further study of the variations of potassium in blood corpuscles. It was proposed therefore to investigate first whether conditions other than repeated hemorrhage might cause changes in the concentration of potassium in the corpuscles. A comparison of the conditions causing the changes should determine whether increases in concentration are due to a storage of potassium in a non-diffusible form, or whether potassium diffuses freely through the corpuscle membrane, shifting in and out as certain investigators in the past believed.

Hamburger (1916) believed that there was a transfer of base between serum and corpuscles due to changes in CO_2 tension. J. Mellanby and Wood (1923), and Collip (1921), came to the same conclusion from their analyses of ashed serum. Doisy and Eaton (1921), however, showed that these shifts of base were not real, but appeared because of the failure of previous investigators to correct for the water shift between serum and corpuscles. Van Slyke, Wu, and McLean (1923) in their presentation of the relationships between corpuscles and serum have assumed that the corpuscles are impermeable to sodium and potassium.

A disturbance of the normal value for potassium in the serum would appear to be the most favorable condition for producing a similar change in the corpuscles. The obvious method for accomplishing this would seem to be to inject a potassium salt into the blood stream and subsequently examine the corpuscles for an increase in potassium. Whelan (1925) found no change in the cells under these conditions. As a trial experiment to test her findings, serum containing an added excess of potassium chloride was added to corpuscles in a test-tube. After 1 hour the corpuscles were separated and analyzed. No change in potassium was found.

In order to study the effect of lowering or raising the level of potassium in the plasma on the corpuscle composition, three groups of experiments were undertaken.

1. Gelatin-saline solution was injected intravenously into dogs in order to lower the plasma potassium by dilution of the blood. The gelatin was added to prevent loss of the injected fluid through the capillary walls (Bayliss, 1915–17).

2. The potassium concentration of plasma was lowered by overdosage of insulin. This effect of insulin was observed by Briggs, Koechig, Doisy, and Weber (1923–24) and by Harrop and Benedict (1924).

3. Dogs were given subcutaneous injections of sodium oxalate. Gross (1923) had found that injection of oxalate caused the concentration of potassium in whole blood to rise to an abnormally high level. Since he did not determine whether this change occurred in serum or in corpuscles, it seemed important to determine the potassium distribution.

Methods.

All of the experiments were conducted on dogs. Blood for analysis was studied throughout in the manner described in the preceding paper (Kerr, 1926). The analytical methods likewise were the same as previously used. Experience gained in the determination of potassium and sodium made certain the detection of small changes in concentration. Glucose was determined by the method of Folin and Wu (1919), inorganic and hydrolyzable phosphorus by the method of Briggs (1924).

EXPERIMENTAL.

Effect of Diluting the Blood with Gelatin-Saline Solution.

In order to avoid the plethora which would result from the injection of a large quantity of diluting fluid into the blood, the injection was in each case preceded by the withdrawal from the heart of a volume of blood equal to the amount of fluid to be injected. Three experiments of this kind were conducted (Experiments 1-G, 2-G, and 3-G, Tables I, II, and III), on fasting dogs.

In the first experiment (Table I), approximately half the blood of the dog was removed rapidly from the heart and jugular vein. Immediately after the withdrawal of the blood an equal volume of 0.95 per cent sodium chloride solution containing 6 per cent gelatin was injected slowly into the jugular vein, the injection requiring about half an hour. Samples were drawn from the heart for analysis 5 minutes after the completion of the injection to study the immediate effects, again 5½ hours later, and finally 24 hours after the injection of gelatin. 5 minutes after the injection, the effect of addition of the saline is evident in the increased serum sodium and chloride, and the lowered potassium, the latter being merely a result of dilution. The absence of a drop in calcium is due to the fact that the gelatin, as shown by analysis, contained calcium. In the corpuscles there was a slight increase in potassium. After 5½ hours sodium, chloride, and potassium have returned to normal levels in serum, and remained so at the 24 hour period. There were no changes in the corpuscles at the 5½ hour period, but 24 hours after the injection there was a great increase in the cell potassium, the concentration more than doubling.

The interpretation of this change in potassium is obscured by two facts—the presence of the gelatin and the three losses of blood when large samples were taken for analysis. The original purpose of the experiment—to determine whether a dilution of the serum potassium would cause a *fall* in cell potassium—was accomplished in that no fall was observed. The subsequent rise, interpreted in the light of the high cell potassium found after repeated hemorrhage, might be due either to the presence of the gelatin, or to the repeated hemorrhage.

A second experiment (Table II) was undertaken in which the initial hemorrhage was purposely made a large one, about 40

TABLE I.

Experiment 1-G. Effect of Hemorrhage, Followed by Intravenous Injection of Gelatin-Saline Solution (6 Per Cent Gelatin in 0.95 Per Cent NaCl).

Dog 3, female; weight 11.4 kilos. Calculated blood volume 912 cc.

Sample No.	Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Weight of cells / Weight of blood ×100.	Remarks.	Analyses.		Sample I.	Sample II.	Sample III.	Sample IV.
			cc.									
I	Feb. 21	10 a.m.	200	22	43	Normal blood sample after 2 day fast. Fed after this sample, but fasted through remainder of experiment.	Sp. gr.	Whole blood.	1.053	1.030	1.031	1.030
								Serum.	1.022	1.021	1.019	1.020
							H_2O, per cent.	Whole blood.	81.7	91.2	90.5	90.1
								Serum.	93.6	94.6	95.2	94.5
								Cells.	66.1	66.5	66.0	66.5
	" 23	10.45 a.m.	440	48		Hemorrhage.	pH (electrometric).	Serum.	7.3	7.3	7.4	7.3
	" 23	10.45–11.30 a.m.				Gelatin-saline solution (440 cc.) injected.	CO_2 mM per liter.	"	19.6	13.8	12.2	14.6
II	" 23	11.35 a.m.	200	22	12		Na " " 1000 gm.	Serum.	136	144	136	138
								Cells.	111	109	110	108

							K mM per 1000 gm.	Serum.	5.8	3 8	5 0	5 3
								Cells.	7.1	8.5	8.1	17.7
							Ca " " 1000 "	Serum.	2.8	3.0	2.5	2.0
III	Feb. 23	5 p.m.	200	22	16		Mg " " 1000 "	"	0.7	0.6	0.6	0.6
							Cl " " 1000 "	Whole blood.	87	108	104	100
								Serum.	112	118	112	107
								Cells.	56	33	61	58
IV	" 24	11 a.m.	200	22	16							
	" 24	2 p.m.				Found dead.						

TABLE II.

Experiment 2-G. Effect of Hemorrhage, Followed by Intravenous Injection of Gelatin-Saline Solution (3 Per Cent Gelatin in 0.80 Per Cent NaCl Solution).

Dog 9, male; weight 6.4 kilos. Calculated blood volume 512 cc. Received water daily.

Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Volume of cells / Volume of blood × 100.	Remarks.	Serum.		Corpuscles.	
						H_2O	K	H_2O	K
		cc.				*per cent*	*mM per 1000 gm.*	*per cent*	*mM per 1000 gm.*
					Last fed Aug. 8; fasted throughout experiment.				
Aug. 10	11 a.m.	200	39	33		93.2	4.7	66.2	7.3
" 10	11.15–11.45 a.m.				Injected 200 cc. gelatin-saline into jugular vein.				
" 11	11.30 a.m.	80	16	25		93.7	3.9	66.6	9.3

TABLE III.

Experiment 3-G. Effect of Hemorrhage, Followed by Intravenous Injection of Gelatin-Saline Solution (9 Per Cent Gelatin in 0.85 Per Cent NaCl Solution.)

Dog. 10, female; weight 8.8 kilos. Calculated blood volume 704 cc. Received water daily.

Date.	Hour.	Volume of blood taken.	Per cent of total blood.	Volume of cells / Volume of blood × 100.	Remarks.	Serum.		Corpuscles.	
						H_2O	K	H_2O	K
		cc.				*per cent*	*mM per 1000 gm.*	*per cent*	*mM per 1000 gm.*
					Last fed Aug. 20; fasted throughout experiment.				
Aug. 24	10.30 a.m.	200	28	42		92.5	5.0	66.5	8.2
" 24	11–11.30 a.m.				Injected 175 cc. gelatin-saline into jugular vein.				
" 25	10.30 a.m.	100	14	28		93.5	5.9	66.4	8.3

per cent of the total blood volume, the blood thus lost being replaced by an equal volume of 3 per cent gelatin in 0.80 per cent sodium chloride. The next blood sample was taken 24 hours later, so that changes could not be ascribed to repeated hemorrhages. Examination of Table II shows that the potassium concentration of the corpuscles increased considerably, while the potassium in serum fell. The fall in hematocrit reading equaled that calculated from the blood lost, indicating no regeneration of red cells. Since single hemorrhages without gelatin injection produced no such effects, the rise in potassium would seem to be due to the gelatin.

A third experiment (Table III) was conducted with the purpose of introduding a large quantity of gelatin into the blood stream with as small a withdrawal of blood as possible. After a hemorrhage amounting to 28 per cent of the total blood volume, a 9 per cent solution of gelatin in 0.85 per cent sodium chloride was introduced intravenously, giving a concentration of about 2.3 per cent gelatin in the blood stream. 24 hours later there was found a small rise in the potassium concentration of the serum but no change in the cells.

That gelatin causes changes in the blood can hardly be doubted, for the arterial blood even 5 minutes after the injection was of a dark color, and the corpuscles settled with great rapidity after defibrination. Hanzlik, De Eds, and Tainter (1923) have also reported this change in color and sedimentation after gelatin and gum acacia injections.

These experiments enable us to conclude only that heavy bleeding followed by injections of gelatin-saline cause the potassium concentration of corpuscles to increase. It remains uncertain whether the change in serum protein accounts for this altered distribution of potassium.

Effect of Insulin.

In order to produce a lowered serum potassium and to study its effect on the concentration of potassium in the corpuscle, dogs were given large doses of insulin. Briggs, Koechig, Doisy, and Weber (1923–24) observed that large doses of insulin caused a definite decrease in serum potassium and inorganic phosphate before the onset of convulsions. Their few analyses on whole blood led them

TABLE IV.

Experiment 1-Ins. Effect of Insulin.

Dog 10, female; weight 8.8 kilos. Calculated blood volume 704 cc.

Date.	Hour.	Volume of blood taken.	Volume of cells / Volume of blood × 100.	Insulin injected, clinical units.	Remarks.	Serum.					Corpuscles.		
						H_2O	Glucose.	Inorganic P.	K	Protein.	H_2O	K	Inorganic + hydrolyzable P.
		cc.				*per cent*	*mg. per 100 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*	*per cent*	*per cent*	*mM per 1000 gm.*	*mM per 1000 gm.*
					Last fed 20 hrs. before experiment.								
Aug. 13	10.25 a.m.	70	43		Normal blood sample.	92.7	120	1.3	5.2	6.1	66.6	7.4	4.5
" 13	10.35 "			20									
" 13	11.35 "			10									
" 13	12.20 p.m.			10									
" 13	1.15 "	70	40		Dog depressed but no signs of hyperirritability.	93.4	40	0.55	3.5	5.3	66.1	8.4	4.0

TABLE V.

Experiment 2-Ins. Effect of Insulin.

Dog 11, male; weight 7.3 kilos. Calculated blood volume 584 cc.

Date.	Hour.	Volume of blood taken.	Volume of cells / Volume of blood × 100.	Insulin injected, clinical units.	Remarks.	Serum.					Corpuscles.	
						H_2O	Glucose.	Inorganic P.	K	Protein.	H_2O	K
		cc.				*per cent*	*mg. per 100 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*	*per cent*	*per cent*	*mM per 1000 gm.*
Aug. 17	10.15 a.m.	60	41		Last fed Aug. 14. Normal blood sample.	92.6	143	1.9	3.9	6.1	66.8	9.6
" 17	10.20 "			40								
" 17	12.30 p.m.			30								
" 17	1.50 "			30								
" 17	3.00 "				Dog begins to appear drowsy.							
" 17	3.05 "	50	35			93.4	46	1.1	2.8	5.3	67.5	10.3

TABLE VI.

Experiment 3-Ins. Effect of Insulin.

Dog 12, female; weight 8.4 kilos. Calculated blood volume 672 cc.

Date.	Hour.	Volume of blood taken.	Volume of cells / Volume of blood × 100.	Insulin injected, clinical units.	Remarks.	Serum.					Corpuscles.	
						H_2O	Glucose.	Inorganic P.	K	Protein.	H_2O	K
		cc.				*per cent*	*mg. per 100 gm.*	*mM per 1000 gm.*	*mM per 1000 gm.*	*per cent*	*per cent*	*mM per 1000 gm.*
					Last fed Aug. 21.							
Aug. 24	10 a.m.	70	38		Normal blood sample.	92.4	130	1.5	5.6	6.4	65.8	10.4
" 24	10.05 a.m.			70	Injected insulin subcutaneously. The dog went into convulsions within 1 min. after the insulin. Recovered somewhat in 15 min., but lacked sense of balance.							
" 24	12 m.				Much improved, but not normal.							
" 24	1.30 p.m.	70	38		Blood sample. Dog is depressed, but no return of convulsions.	93.2	75	1.6	5.7	5.8	67.2	12.7

to state that there seemed to be a loss of potassium from the corpuscles as well as from serum. Harrop and Benedict (1924), studying the effect of insulin upon human diabetics and on normal rabbits, found that potassium, inorganic phosphates, and glucose in serum decreased after insulin, the potassium dropping to as much as half of its original level. Blood samples were taken before the injections, and again 2 or 3 hours afterwards, during the period of depression preceding the onset of hyperirritability. The details of procedure and the analytical results are presented in Tables IV, V, and VI.

In spite of the large doses of insulin injected, the first two dogs showed no signs of hyperirritability, although they were uneasy and depressed. The third dog went into convulsions within less than a minute after the injection. This could not have resulted so quickly from the hormone injection.

In the first two experiments, the fall in serum glucose and in inorganic phosphate was accompanied by a marked decrease in serum potassium. In both cases there was a simultaneous *rise* in the potassium content of the corpuscles. In the third experiment, which was complicated by convulsions from an unknown cause, there was no change in serum potassium or phosphate, and glucose did not fall to a low level. In the corpuscles, however, there was a great increase in potassium concentration. In the insulin experiments the increased concentration of potassium in the corpuscles was usually accompanied by an increase in the water content of the corpuscles. Correction for this water shift shows that the transfer of potassium into the cells is even greater than the table indicates. In each of the three experiments there was a fall in serum proteins after the insulin injections. This may have been due to withdrawal of the fairly large blood samples immediately before the insulin injections, since the amount taken was nearly 10 per cent of the total blood volume. It is quite possible, however, that the fall in serum proteins was a direct result of the insulin, in which case it may be of significance in connection with the changed potassium distribution.

Effect of Oxalate Poisoning.

Gross (1923) studied the effects of injections of sodium oxalate on the inorganic constituents of the whole blood of dogs. He

TABLE VII.

Effect of Sodium Oxalate Injections.

Dog 8, female; weight 15.3 kilos. Fasted 1 week before experiment. Received water daily. Calculated blood volume, 1224 cc.

Sample No.	Date.	Hour.	Volume of blood taken.	Weight of cells / Weight of blood × 100.	Remarks.	Analyses.		Sample I.	Sample II.
			cc.		Fasted July 22-31.				
I	July 29	10 a.m.	100	51	Blood sample.	H_2O, per cent.	Whole blood.	78.8	81.4
							Serum.	92.2	93.4
							Cells.	66.3	65.2
	" 29	4 p.m.			Injected 1 gm. sodium oxalate in 30 cc. H_2O.	Na mM per 1000 gm.	Serum.	135	135
							Cells.	114	115
	" 30	11 a.m.			Injected 1 gm. sodium oxalate in 30 cc. H_2O.	K " " 1000 "	Serum.	5.0	9.7
							Cells.	8.9	11.3
	" 30	4.30 p.m.			Injected 0.66 gm. sodium oxalate in 20 cc. H_2O. No signs of tetany.	Ca " " 1000 "	Serum.	3.1	2.2
						Mg " " 1000 "	"	1.3	1.2
	" 31	10 a.m.			Injected 1 gm. sodium oxalate in 30 cc. H_2O. No tetany.	Cl " " 1000 "	Whole blood.	76	62
							Serum.	105	88
							Cells.	49	27
II	" 31	1 p.m.	125	42	Blood sample.	P (inorganic) mM per 1000 gm.	Serum.	1.1	4.3
							Cells.	1.9	1.7
	" 31	3 "			Died. No tetany.	Protein, per cent.	Serum.	6.7	5.6

found a great increase in potassium and inorganic phosphate, with a decrease in calcium, sodium, and chloride. The distribution of these elements between corpuscles and serum was not investigated.

A single experiment was therefore carried out to determine whether the changes noted by Gross involved changes in the corpuscles as well as in the serum. The results are given in Table VII. Although the dosage of oxalate injected was about the maximum used by Gross, the dog did not develop tetany, serum calcium falling only to 2.2 mM per 1000 gm. Sodium remained unchanged in both serum and cells, while chlorides fell to levels much lower than normal. The potassium concentration in serum almost doubled, and in corpuscles there was an unmistakable rise. The rise in cell potassium appears to be smaller than in serum, but when the concentrations are stated in terms of millimols per kilo of water it is seen that the cells have shared almost equally in the increase. The decrease in water content of the corpuscles accounts for only 0.2 mM of the increase in potassium. Inorganic phosphates in serum increased remarkably. Serum proteins decreased to a considerable extent. The changes most significant for the purposes of this paper are the great increases of both potassium and inorganic phosphate in the serum, and of potassium in the cells. The change in serum proteins may be of importance in influencing the new potassium distribution.

DISCUSSION.

In each of these three groups of experiments, the changes in the concentration of potassium in the corpuscles are of such a nature that there can be little doubt that potassium actually passes from the plasma into the cells. After insulin injections the changes are so rapid that the potassium concentration in the corpuscles rises within 2 hours. Doisy and Eaton (1921) have shown that potassium does not shift in and out of corpuscles due to changes in carbon dioxide tension. As the change after oxalate injection involves an equimolar increase in both cell water and serum water, the obvious inference is that free diffusion may have occurred, but it should be noted that both before and after injection the concentration of potassium in the cell water is higher than in serum water, so that some other explanation must be considered. Since the

potassium concentration may increase in the cells while it increases, decreases, or remains unchanged in the serum, the conclusion seems inevitable that potassium may under some conditions enter the corpuscle readily and be stored in a non-diffusible form. Changes in serum proteins and inorganic phosphates may however be of significance.

SUMMARY AND CONCLUSIONS.

1. Changes in the potassium concentration of serum and corpuscles were studied.

2. A single large hemorrhage followed by gelatin-saline infusion caused the potassium concentration of the corpuscles to increase.

3. Overdosage with insulin resulted in a fall of serum potassium and a rise of cell potassium.

4. Subcutaneous injections of sodium oxalate caused a large increase in potassium of both serum and corpuscles.

5. The changes in potassium concentration of the corpuscles cannot be accounted for by shifts of water, but only by an actual penetration into the cell by potassium.

6. It would appear that, though the red corpuscle is permeable to potassium, it must be combined to some extent in non-diffusible form.

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